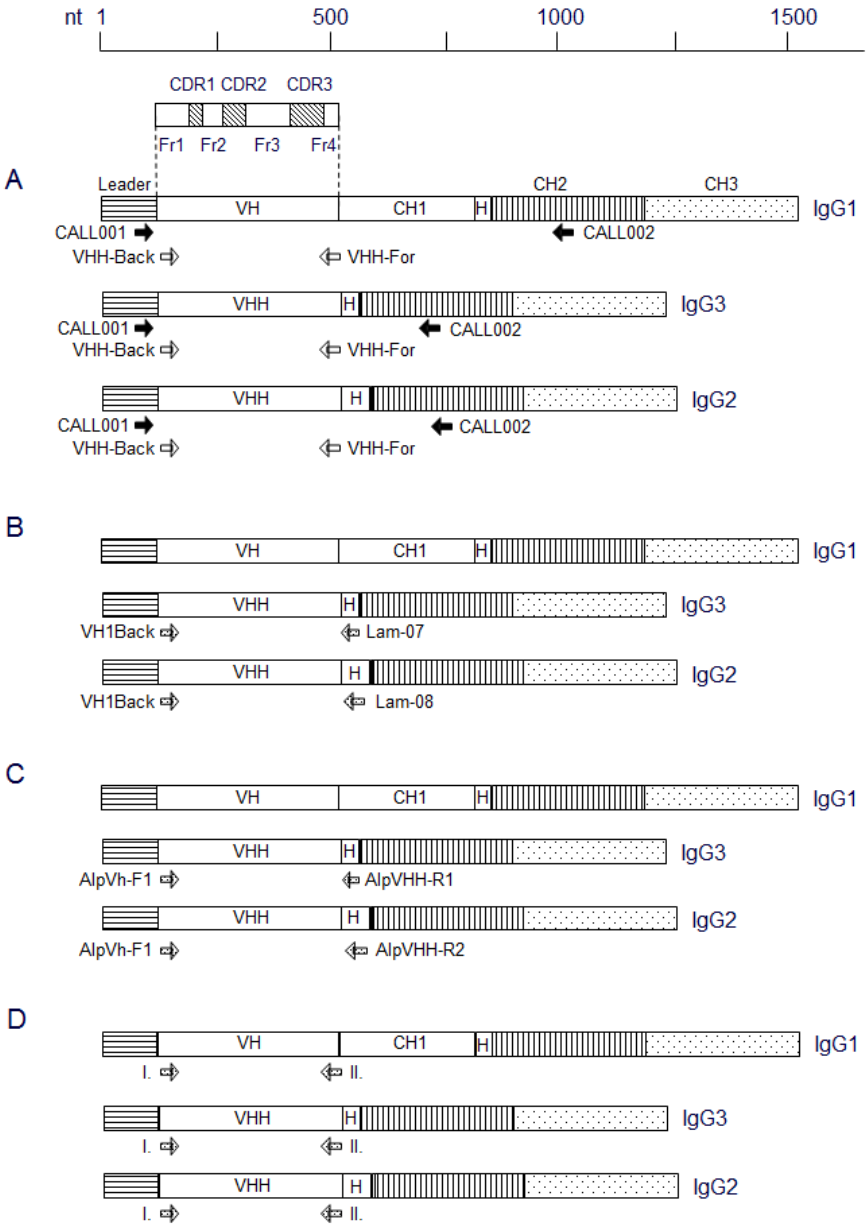
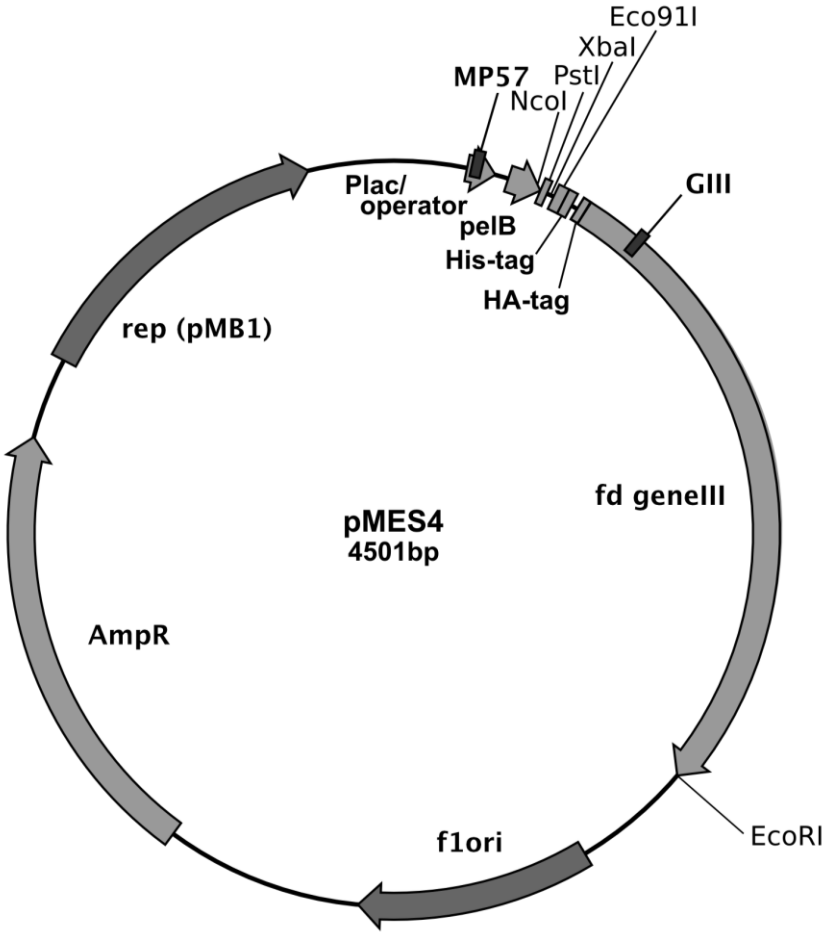



**Figure S1**| Strategies to amplify the Nanobody repertoire by PCR from PBL cDNA. For each primer, we specify the locus of hybridization on the cDNAs encoding the heavy chains of the conventional antibodies (isotype IgG1), on the cDNAs encoding the heavy chain only antibodies of isotype IgG3 or on the cDNAs encoding the heavy chain only antibodies of isotype IgG2. Arrows representing the primers are not drawn to scale. H: hinge; CH2: constant domain 2; CH3: constant domain 3; Fr1 (to 4): framework 1 (to 4); CDR1 (to 3): complementarity determining region 1 (to 3); VH: variable domain of the heavy chain of a conventional antibody (IgG1); VHH: variable domain of a heavy chain only antibody (IgG2 or IgG3). Immunoglobulin domains with highly conserved DNA sequences amongst camelid species (leader, CH2, CH3) are pattern filled. (A) Primers CALL001 and CAL002 to amplify the variable domains of all camelid immunoglobulin heavy chains from PBL cDNA (Step 21) are depicted in black. The primers VHH-Back and VHH-For to amplify the Nanobody repertoire via nested PCR (Step 24) are depicted with open arrows. (B) Van der linden *et al*. developed dedicated primers to separately amplify the IgG2 and IgG3 isotypes from *llama glama*[49] (C) Maass *et al*. developed primers to separately amplify the IgG2 and IgG3 isotypes from *llama pacos*[50] (D) Kastelic *et al*. developed a set of primers that amplify all VHs and VHHs from llama[51].

A




B

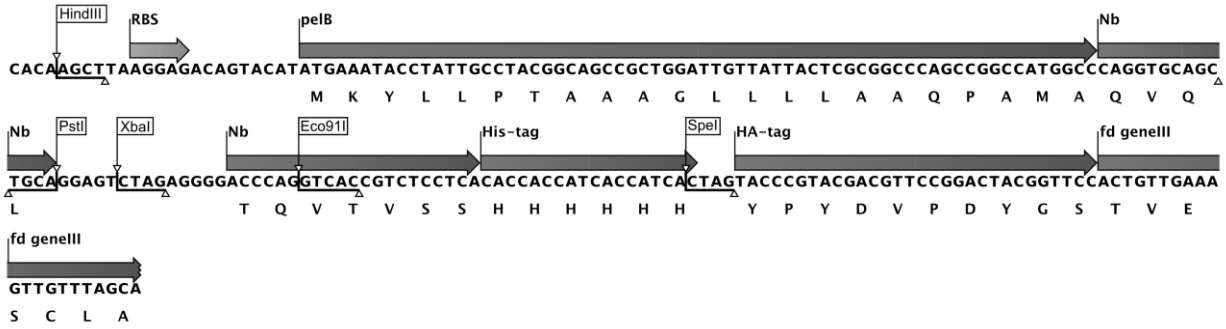
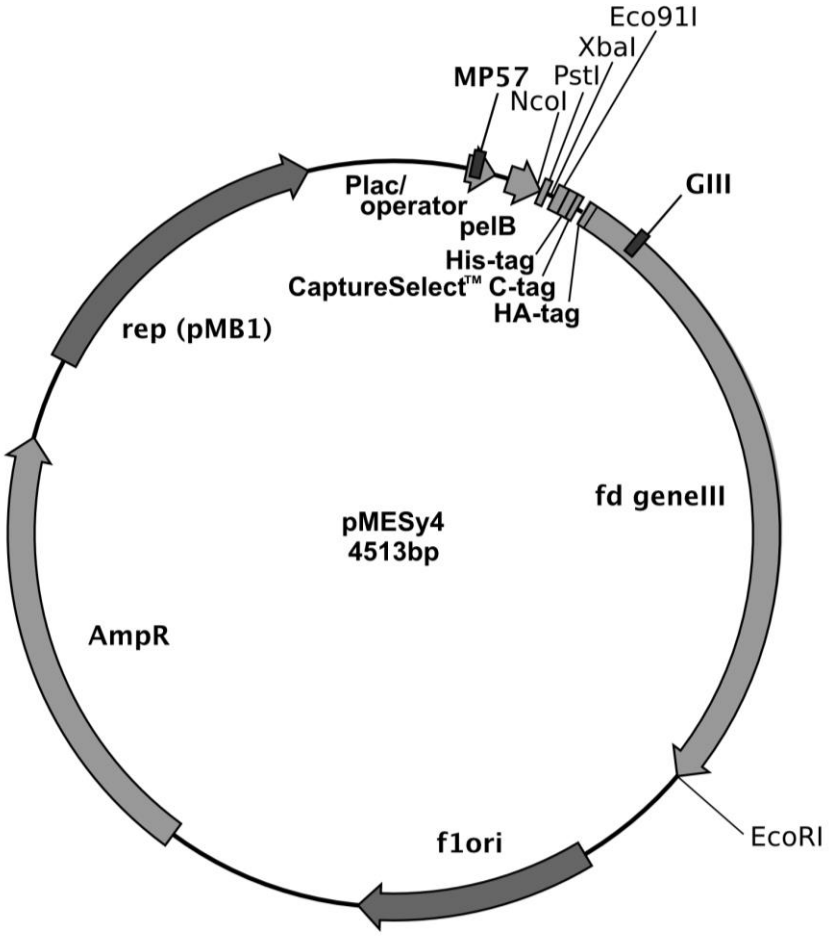



**Figure S2|** Phage display vector pMES4. A. Map of pMES4 (4501bp, Genbank GQ907248). Nanobodies can be cloned as PstI-Eco91I fragments (step 24) in between the *pel*B sequence (pelB) coding for the secretion signal peptide of PelB and a 6xHis-tag (His-tag) followed by the hemagglutinin tag (HA-tag) and gene III of filamentous phage fd (fd geneIII). Other annotations are the *lac* promotor/operator (Plac/operator), the gene conferring ampicillin resistance ($Amp^R$), the bacterial origin of replication (rep) and the f1 origin of replication (f1ori). The annealing sites for the forward sequencing primer (MP57) and the backward primer (GIII) are also indicated on the map (used in Steps 30 & 68). B. Nucleotide sequence and amino acid sequence of the Nanobody cloning site. An amber stopcodon (TAG) is located downstream of the His-tag.

A




B

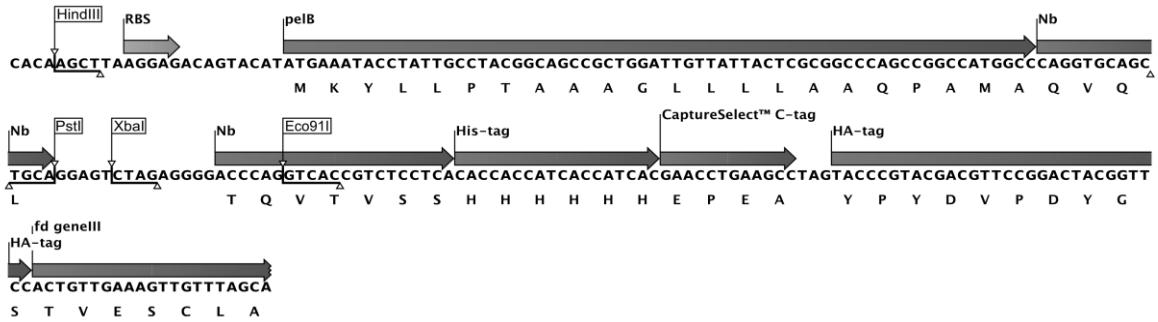



**Figure S3|** Phage display vector pMESy4. A. Map of pMESy4 (4513bp; Genbank KF415192). Nanobodies can be cloned as PstI-Eco91I fragments (step 24) in between the *pel*B sequence (pelB) coding for the secretion signal peptide of PelB and a 6xHis-tag (His-tag), followed by the CaptureSelect™ C-tag, the hemagglutinin tag (HA-tag) and gene III of filamentous phage fd (fd geneIII). Other annotations are the *lac* promotor/operator (Plac/operator), the gene conferring ampicillin resistance ($Amp^R$), the bacterial origin of replication (rep) and the f1 origin of replication (f1ori). The annealing sites for the forward sequencing primer (MP57) and the backward primer (GIII) are also indicated on the map (used in Steps 30 & 68). B. Nucleotide sequence and amino acid sequence of the Nanobody cloning site. An amber stopcodon (TAG) is located downstream of the CaptureSelect™ C-tag.